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The nuclear receptor CAR modulates alcohol-induced liver injury

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The constitutive androstane receptor (CAR) is a member of the nuclear receptor superfamily and a sensor and detoxifier of both xenobiotics and endobiotics. Recent studies also show that CAR participates in metabolism of glucose and lipid, and has an important role in fatty liver disease and diabetes. In this study, we investigate the roles of CAR in chronic and acute alcohol-induced liver injuries. The results showed that absence of CAR in rodents led to significantly increased susceptibility to chronic alcohol-induced liver injury, which was accompanied with elevated hepatocyte apoptosis and fat accumulation. However, pre-activation of CAR by a CAR agonist, TCPOBOP, strongly enhanced the hepatic toxicity by both chronic and acute alcohol infusion in wild-type, but not in $CAR^{-/-}$ mice. Gene expression analyses indicated that CAR pre-activation and alcohol infusion synergistically decreased the expression of enzymes that metabolize the alcohol in liver. These results support a role of CAR in modulating alcoholic liver injury and imply a risk of synergistic liver toxicity induced by alcohol and CAR activation.

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KEYWORDS: alcohol; alcohol dehydrogenase; aldehyde dehydrogenase; constitutive androstane receptor; liver injury

Alcohol-induced liver injury is one of the major causes of liver diseases in Western countries. Several mechanisms of alcoholic liver diseases have been uncovered and summarized.[1,2] Metabolism of alcohol generates acetaldehyde and converts NAD + to NADH. Acetaldehyde is toxic to liver and the higher ratio of NADH/NAD + disrupts the normal lipid metabolism in liver, thereby leading to fatty liver diseases. Alcohol-induced liver injury is tightly associated with hepatocyte deaths, steatosis, hepatitis, and fibrogenesis. However, the exact mechanisms by which alcohol interrupts normal liver metabolism and causes liver injury are still unclear.

The constitutive androstane receptor (CAR, NR1l3) is a member of the nuclear receptor superfamily. CAR functions together with the pregnane X receptor (PXR, NR1l2) as sensors and detoxifiers for endobiotics and xenobiotics by regulating their metabolism and excretion.[3] CAR can be activated by phenobarbital and other structurally phenobarbital-like molecules.[4] The major target genes of CAR include cytochrome P450 enzymes such as CYP1A2, CYP2E1, CYP2B10, and CYP3A11.[5] Recent studies also reported that CAR has a role in diabetes mellitus and metabolism of glucose and lipid.[6,7] On the other hand, several nuclear receptors, PPARα, PPARγ, PPARδ, RAR, and HNF4α, have been shown to be involved in alcohol-induced liver injury.[8–12] Considering the importance of CAR in P450 enzyme expression and metabolisms of glucose and lipids, we ask whether CAR has a role in modulating alcohol-induced liver injury. In this study, we compared both chronic and acute alcohol-induced liver injury in $CAR^{-/-}$ and wild-type mice. We also applied a potent CAR activator, 1,4-bis (2-(3,5-dichloropyridyloxy))benzene (TCPOBOP), to pre-activate CAR and evaluated the roles of CAR in modulating alcohol-induced liver injury.

MATERIALS AND METHODS

Chronic Alcohol-induced Liver Injury Rodent Models

$CAR^{-/-}$ mice and wild-type mice on a C57BL/6 background were maintained in the animal resource facility of the City of Hope National Medical Center. The mice were housed in a temperature- and light-controlled room (25°C; 12-h light/

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dark cycle), and fed with standard rodent chow and water ad libitum. For the chronic alcohol-induced liver injury model, the $CAR^{-/-}$ mice and the wild-type mice were fed with an ethanol-containing or control rodent liquid Lieber-DeCarli '82 diet described previously.[8] The liquid diet was ordered from Bio-serv (Frenchtown, NJ, USA), and prepared and maintained as manufacturer's protocols. Because $CAR^{-/-}$ mice are more susceptible to diabetes,[6,7] the maltose in the original recipe was replaced with isocaloric liquid-diet powder. Ethanol concentration was increased gradually from 1 to 5% within 4 weeks. For the control diet, additional diet powder was added to the diet recipe to provide equal calories with ethanol. The mice had free access to the liquid diet and the average consumption was 12 ± 1 ml ethanol or control liquid diet per day per mouse for both the $CAR^{-/-}$ mice and the wild-type mice. The feeding started when the mice were 8 weeks old, and TCPOBOP was applied with a dose of 3 mg/kg by i.p. injection 24 h before the ethanol-containing diet feeding was started. The mice were injected by TCPOBOP every 2 weeks and seven times in total.

Acute Alcohol-induced Liver Injury Rodent Models

The mice were pretreated with 3 mg/kg of TCPOBOP by i.p. injection once a day for 3 days before the oral infusion of ethanol. At 24 h after the last injection of TCPOBOP, 2.5 g/kg of ethanol was injected i.p. into the mice every 12 h for six times. At 24 h after the last injection of ethanol, the mice were culled and their livers and serum were harvested.

Staining

Small pieces of tissue from the right lobe of the livers were fixed in 4% PBS-buffered paraformalin. The livers were prepared as either paraffin sections or frozen section as described previously.[13,14] The sections were stained with hematotxylin and eosin (H&E) or *in situ* cell death kit (Roche Applied Science, Indianapolis, IN, USA). To quantify the number of cell deaths, six fields were randomly chosen from each liver section. The Oil-Red O staining was performed according to a previous publication.[15]

Message RNA Analysis

The total RNAs were prepared with TRI-reagent (Molecular Research Center, Cincinnati, OH, USA). First-strand cDNA was synthesized from the total RNAs using Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen, San Diego, CA, USA). The gene expression was quantified by real-time quantitative PCR using an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Forest City, CA, USA). The specific forward (F) and reverse primers (R) were provided in Table 1. The quantity of mRNA was normalized by internal standard mouse acidic ribosomal phosphoprotein P0 m36b4.

Western Blot

Western blot was performed as previously described.[14] Anti-ADH and anti-aldehyde dehydrogenase (ALDH) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-β-actin antibody was from Sigma (Santa Louis, MO, USA).

Lipid Peroxide, Aspartate Aminotransferase, and Alanine Aminotransferase Analysis

Liver lipid peroxides were measured by a kit from Cayman Chemicals (Ann Arbor, MI, USA). Serum was obtained by centrifuging whole-mouse blood at 3500 r.p.m. at 4°C for 10 min. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured at the City of Hope Helford Research Hospital.

Statistical Analysis

All the data were reported as mean ± s.e.m. Two-tailed Student's *t* test was used to determine the significance of differences between data groups. The *P*-value and the statistical method were indicated individually for each figure.

RESULTS

Chronic Ethanol Treatment Induces More Severe Liver Damage in $CAR^{-/-}$ Mice

Supplement of dietary ethanol for 3 months slightly increased the size of livers in the wild-type mice. However,

Table 1 mRNA analysis

Gene Name	Forward primer	Reverse primer
ADH1	5′-TTACAGGTTAGCTTTTCCCTTCG-3′	5′-TGAATCAGTCTGGTTTAGGGTTCA-3′
ALDH1A1	5′-TTCGGTGGATTCAAGATGTCTG-3′	5′-TCTGCTGGCTTCTTTAGGAGTTC-3′
ALDH3A2	5′-GCTGAAGCAGTTCAACAAAGGA-3′	5′-GGAGAGGCAACAAGGAAGTCAT-3′
CAT	5′-ATCCAGGCTCTTCTGGACAAGT-3′	5′-TTACAGGTTAGCTTTTCCCTTCG-3′
CYP2B10	5′-TTAGTGGAGGAACTGCGGAA-3′	5′-TGAGTGAAAAGGTCTGATAG-3′
CYP2E1	5′-GATTCATCAACCTCGTCCCTTC-3′	5′-TGTCTCTGGATCTGGAAACTCA-3′
SOD1	5′-CTCTCAGGAGAGCATTCCATCA-3′	5′-TGGTTTGAGGGTAGCAGATGAG-3′
SOD2	5′-TGGCTTCAATAAGGAGCAAGGT-3′	5′-GGTAGTAAGCGTGCTCCCACA-3′
SOD3	5′-CTTCACCAGAGGGAAAGAGC-3′	5′-GTCTGCTAGGTCGAAGCTGG-3′

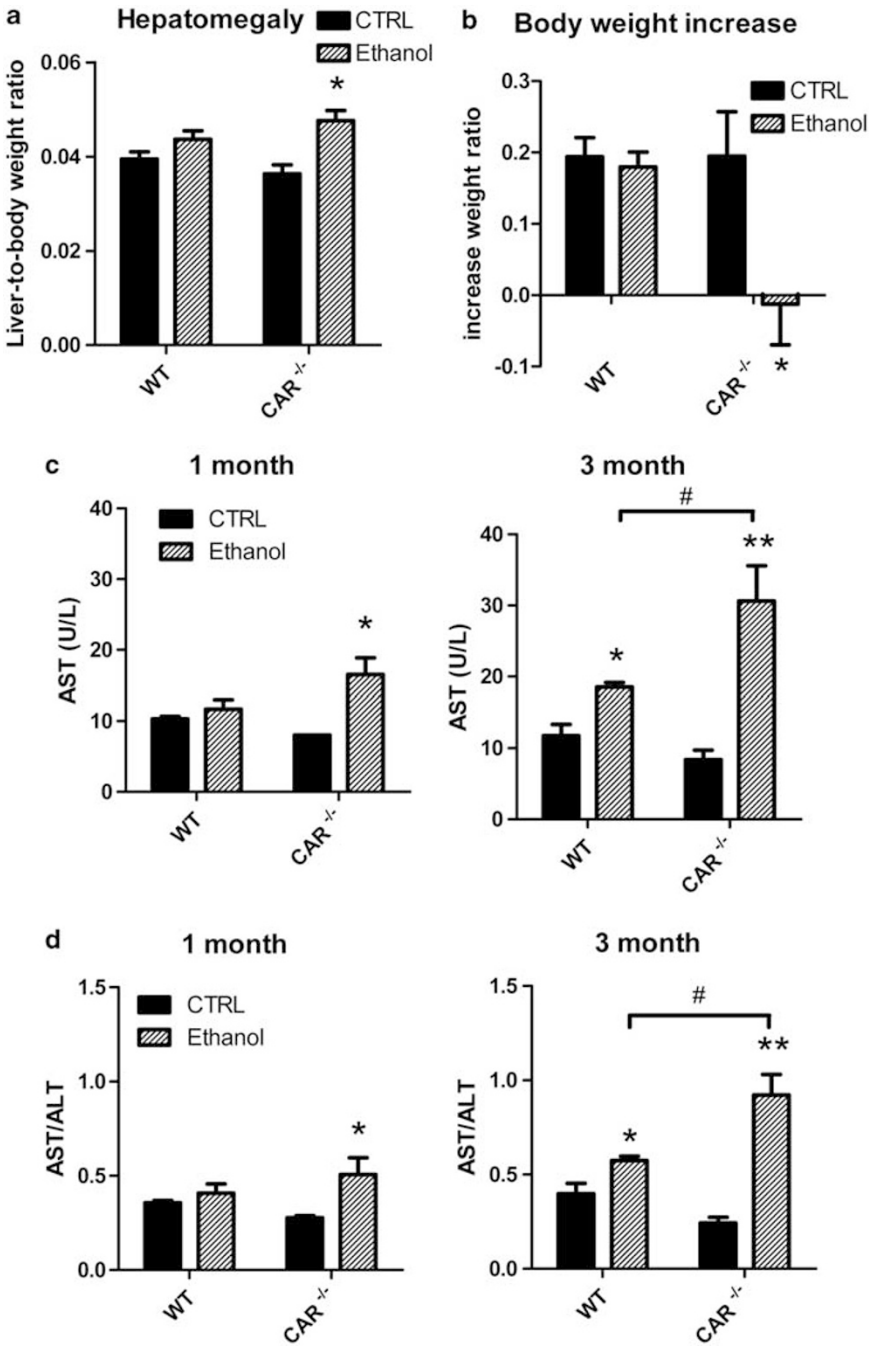

Figure 1 Comparison of ethanol-induced liver injury in the wild-type (WT) and the constitutive androstane receptor ($CAR^{-/-}$) mice. (**a**) Liver-to-body weight ratio after 3 months chronic ethanol-containing diet feeding. (**b**) Body weight increase after 3 months chronic ethanol-containing diet feeding. The body weight of the individual mouse after the treatment is normalized to the original body weight of the same mouse. The number of mice in each group is from 8 to 10. (**c**) Serum aspartate aminotransferase (AST) levels in the mice after 1 month or 3 months ethanol-containing diet feeding. Each group includes results from three or four mice. (**d**) Ratio of serum AST to alanine aminotransferase (ALT). $^{*}P<0.05$, Ethanol *vs* CTRL; $^{**}P<0.01$, Ethanol *vs* CTRL; $^{\#}P<0.05$, $CAR^{-/-}$ *vs* WT.

the same treatment led to significant hepatomegaly in the $CAR^{-/-}$ mice (Figure 1a). The ethanol diet did not impair the body weight increase of wild-type mice after 3 months. In contrast, although the control diet increased the body weight of the $CAR^{-/-}$ mice, the ethanol-containing diet prevented the $CAR^{-/-}$ mice from gaining weight (Figure 1b). To determine whether these alternations resulted from ethanol-induced hepatotoxicity, we compared AST and ALT levels in the wild-type and the $CAR^{-/-}$ mice that were treated with control or the ethanol-containing diet. The ethanol-containing diet, as

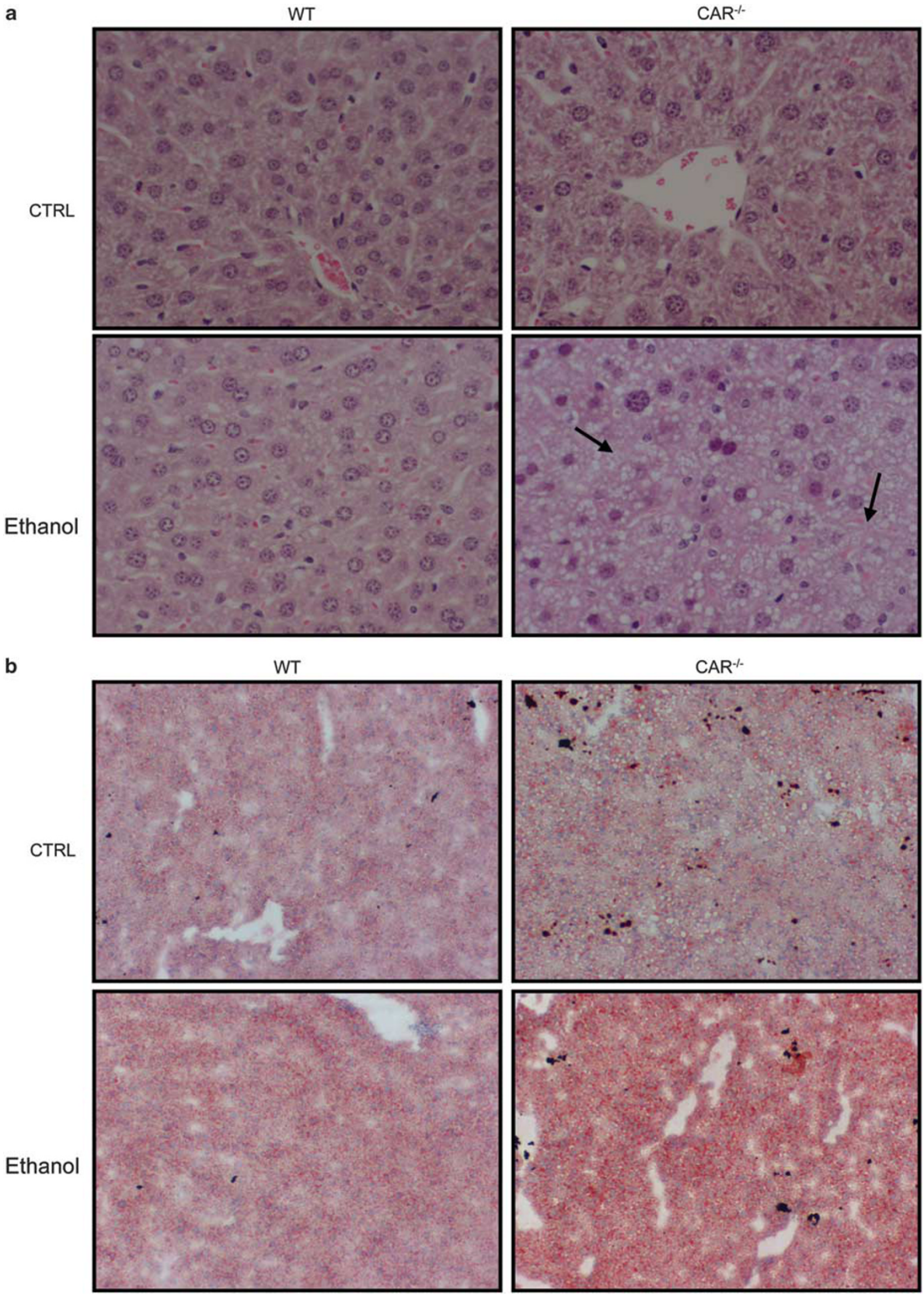

Figure 2 Hepatocyte deaths and fat accumulation resulting from chronic ethanol consumption. (**a**) Representative H&E staining of livers from the mice after 3 months ethanol-diet feeding. Magnification: × 200. Black arrows indicate early signs of necrosis. (**b**) Representative Oil-Red O staining. (**c**) Quantifications of dead cells by TUNEL staining in the livers from the mice after ethanol diet feeding. Each group includes results from five or six mice. $^{*}P<0.05$, Ethanol *vs* CTRL.

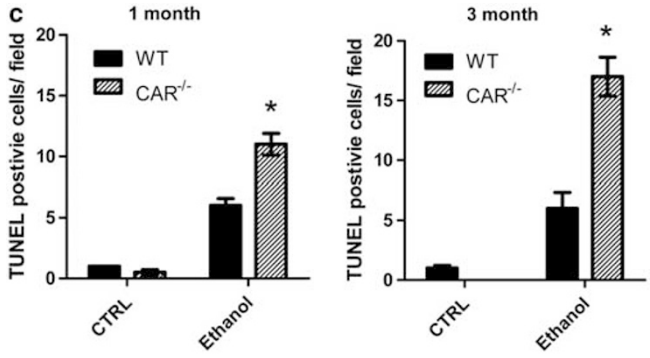

Figure 2 Continued.

expected, elevated AST levels in the wild-type mice after 3 months feeding. However, AST levels were increased as early as 1 month in the $CAR^{-/-}$ mice, and reached a much higher level than those in wild-type mice after 3 months (Figure 1c). On the other hand, ALT levels were comparable between the wild-type and $CAR^{-/-}$ mice (data not shown). This result is consistent with the observations in human patients with moderate chronic alcohol-induced liver injury, in which a higher ratio of AST/ALT is often associated with more severe liver damage. Indeed, the AST/ALT ratio in the $CAR^{-/-}$ mice was much higher than that in the wild-type mice after ethanol feeding either for 1 month or 3 months (Figure 1d).

$CAR^{-/-}$ Mice are More Susceptible to Ethanol-induced Fat Accumulation and Hepatocyte Apoptosis

As CAR activation improves metabolism of lipid and glucose in diabetes mellitus,[6,7] we examined whether the $CAR^{-/-}$ mice would exhibit higher grade of steatosis than the wild-type mice by H&E staining and Oil-Red O staining. The long-term ethanol diet feeding caused minor fat accumulation in the wild-type mice (Figures 2a and b). In the $CAR^{-/-}$ mice, more severe steatosis was observed. In addition, abnormal morphology of hepatocytes was observed in those mice, which indicates potential early signs of necrosis. Chronic alcohol-induced oxidative stress and toxicity are known to result in hepatocyte apoptosis and CAR can promote hepatocyte survival. We therefore compared hepatocyte apoptosis of the wild-type mice and the $CAR^{-/-}$ mice by TUNEL staining. Although the control diet induced steatosis in the $CAR^{-/-}$ mice, it did not cause cell deaths in the wild-type mice or the $CAR^{-/-}$ mice (Figure 2c). However, the ethanol diet-fed $CAR^{-/-}$ mice exhibited more apoptotic cells than the wild-type mice fed with the same ethanol diet, indicating that $CAR^{-/-}$ mice are more susceptible to ethanol-induced hepatocyte apoptosis.

Activation of CAR by TCPOBOP Strongly Enhanced Hepatotoxicity in Chronic Alcohol Feeding

As the $CAR^{-/-}$ mice showed enhanced chronic ethanol-induced liver injury, we asked whether CAR agonists can help to prevent the alcoholic liver damage. Therefore, we pre-treated the mice with a CAR agonist, TCPOBOP, and then fed with 5% alcohol diet by i.p. injections. As expected, TCPOBOP strongly induced the expression of *CYP2B10*, a primary CAR target gene in the wild-type mice but not in the $CAR^{-/-}$ mice (Figure 3a).

However, combined treatment of TCPOBOP and ethanol diet surprisingly led to severe hepatic necrosis shown by H&E staining (Figure 3b) and induced robust increases of AST and ALT levels only in the wild-type mice, but not in the $CAR^{-/-}$ mice (Figures 3c and d). Furthermore, TCPOBOP and the ethanol diet together suppressed the gain of body weight. Though TCPOBOP and ethanol also abolished the body weight increase in the $CAR^{-/-}$ mice, TCPOBOP did not further augment the effects of ethanol diet alone on body weight in these mice (Figure 3e). Moreover, there was a synergistic effect of TCPOBOP and the ethanol diet in reducing the size of liver in the wild-type mice (Figure 3f). In contrast, the ethanol diet resulted in significant hepatomegaly in the $CAR^{-/-}$ mice though TCPOBOP did not further enhance the ethanol-induced hepatomegaly in these mice.

Pre-activation of CAR Enhances Acute Alcohol-induced Liver Injury

To study whether the synergistic effects of TCPOBOP and ethanol can be also seen in acute alcohol-induced liver injury model, we pre-treated mice with TCPOBOP and then injected i.p. high doses of ethanol. This co-treatment caused high death rates in wild-type mice within 24 h post-treatment, whereas ethanol alone did not result in any death (Figure 4a). Consistent with results from chronic alcohol-induced liver injury models, ethanol infusion alone to $CAR^{-/-}$ mice also led to considerable animal deaths, but addition of TCPOBOP to ethanol treatment did not further increase the death rates of the $CAR^{-/-}$ mice, indicating a synergistic effect between ethanol and CAR activation. The high death rate of the wild-type mice treated with both TCPOBOP and ethanol was apparently due to exaggerated liver injury in those mice, which was reflected by the robust ALT induction after TCPOBOP and ethanol co-treatment (Figure 4b). On the other hand, acute high doses of ethanol greatly increased ALT in the $CAR^{-/-}$ mice, but TCPOBOP failed to further enhance this increase. In summary, although basal CAR activity protects liver from ethanol-induced liver injury, pre-activation of CAR, however, greatly enhances ethanol-induced liver injury.

To further examine the liver damage induced by the high dose of alcohol and TCPOBOP, we evaluated the livers from the wild-type mice and the $CAR^{-/-}$ mice by H&E staining and TUNEL staining. The most striking difference was found in the wild-type mice treated with both TCPOBOP and ethanol. TCPOBOP and ethanol co-treatment resulted in the highest number of apoptotic cells in the wild-type mice (Figure 4c). Furthermore, the scattered necrosis seemed in certain areas of their livers, whereas no necrosis was observed

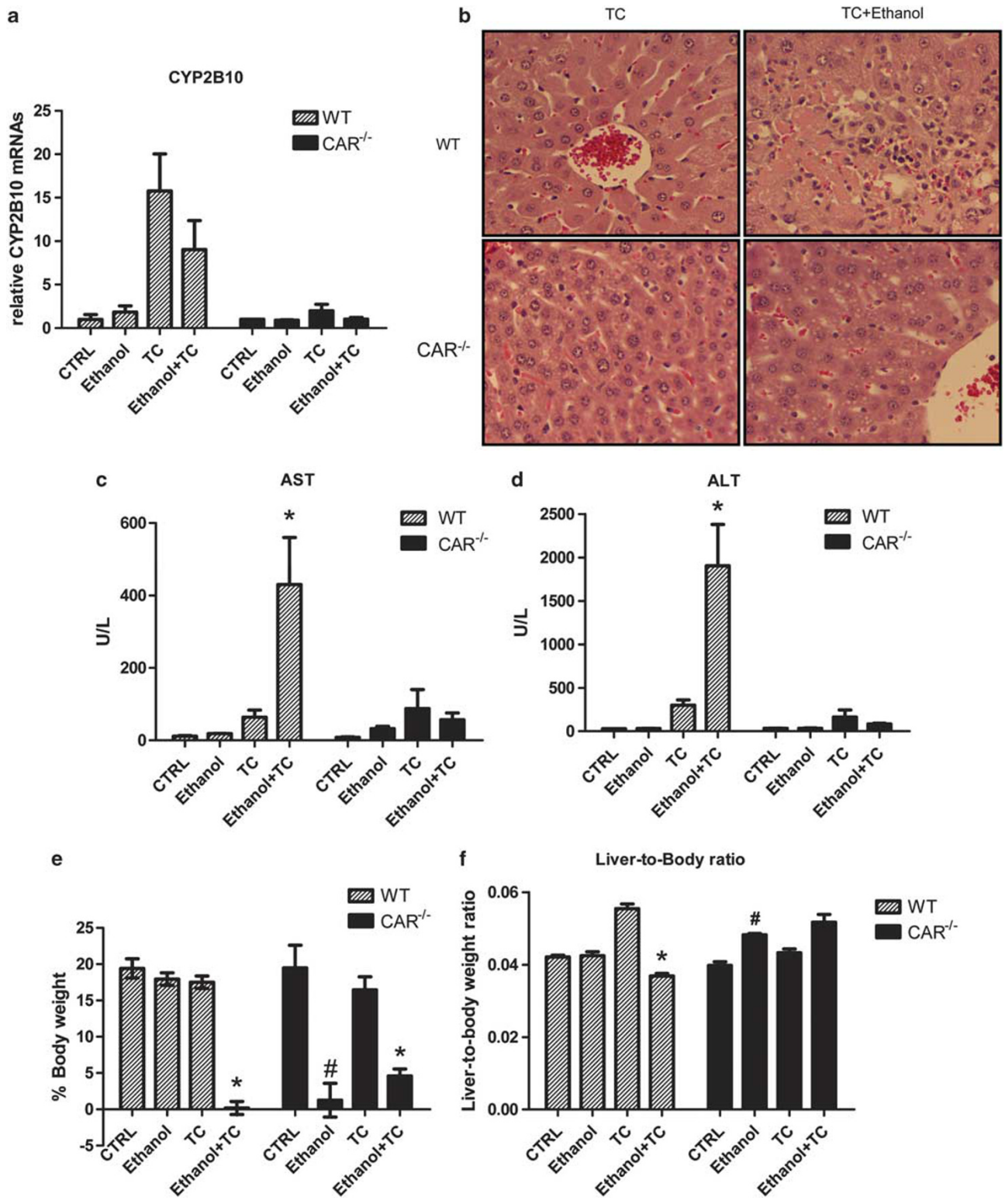

Figure 3 Synergistic effect between TCPOBOP (TC) and chronic ethanol diet feeding on liver injury. (**a**) The liver mRNA levels of CYP2B10 after ethanol and/or TCPOBOP treatments. (**b**) Representative H&E staining of liver sections. Magnification: $\times 200$. (**c** and **d**) Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. (**e**) Body weight increase. (**f**) Liver-to-body weight ratio. Each group includes results from four to six mice. $^{*}P<0.05$, Ethanol + TC *vs* TC. $^{\#}P<0.05$, Ethanol *vs* CTRL.

in the mice of the other groups (Figure 4d). On the other hand, the ethanol-treated $CAR^{-/-}$ mice exhibited massive steatosis, which were also seen in the $CAR^{-/-}$ mice treated with both TCPOBOP and ethanol.

Gene Expression Analyses of Liver Ethanol-metabolizing Enzymes

The hepatic enzymatic systems metabolizing ethanol include alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and CYP2E1. In the cytoplasm of hepatocytes, ethanol is oxidized by ADH to acetaldehyde, which is then further rapidly oxidized to acetate by ALDH. When ADH is saturated by ethanol overload, CYP2E1 joins in the metabolism of ethanol. TCPOBOP treatment led to a significant increase in the expression of *ADH1*, catalase (*CAT*), *ALDH1A1*, and *ALDH3A2* in the wild-type mice but not in the $CAR^{-/-}$ mice, suggesting that CAR may potentially increase the expression of these genes. However, we found that co-treatment of ethanol and TCPOBOP greatly suppressed the expression of ADH1, CAT, ALDH1A1, and ALDH3A2 in liver (Figures 5a and b). This synergistic effect was not observed in the $CAR^{-/-}$ mice. Ethanol alone decreased the expression of these ethanol metabolism genes but TCPOBOP did not further suppress their expression. Liver CYP2E1 was also substantially repressed by TCPOBOP and ethanol co-treatment in the wild-type mice (Figure 5c). However, neither ethanol nor TCPOBOP reduced CYP2E1 expression in the $CAR^{-/-}$ mice. The repression of ADH and ALDH by TCPOBOP and ethanol treatment in the wild-type mice was further confirmed by western blot (Figure 5d).

Ethanol metabolism also generates reactive oxygen species and hydroxyethy radicals, which in turn causes oxidative stress and liver damage. Therefore, we compared the hepatic peroxide levels between the wild-type and the $CAR^{-/-}$ mice. However, there was not a significant difference between the wild-type and the $CAR^{-/-}$ mice with the same treatment (Figure 6a). Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into

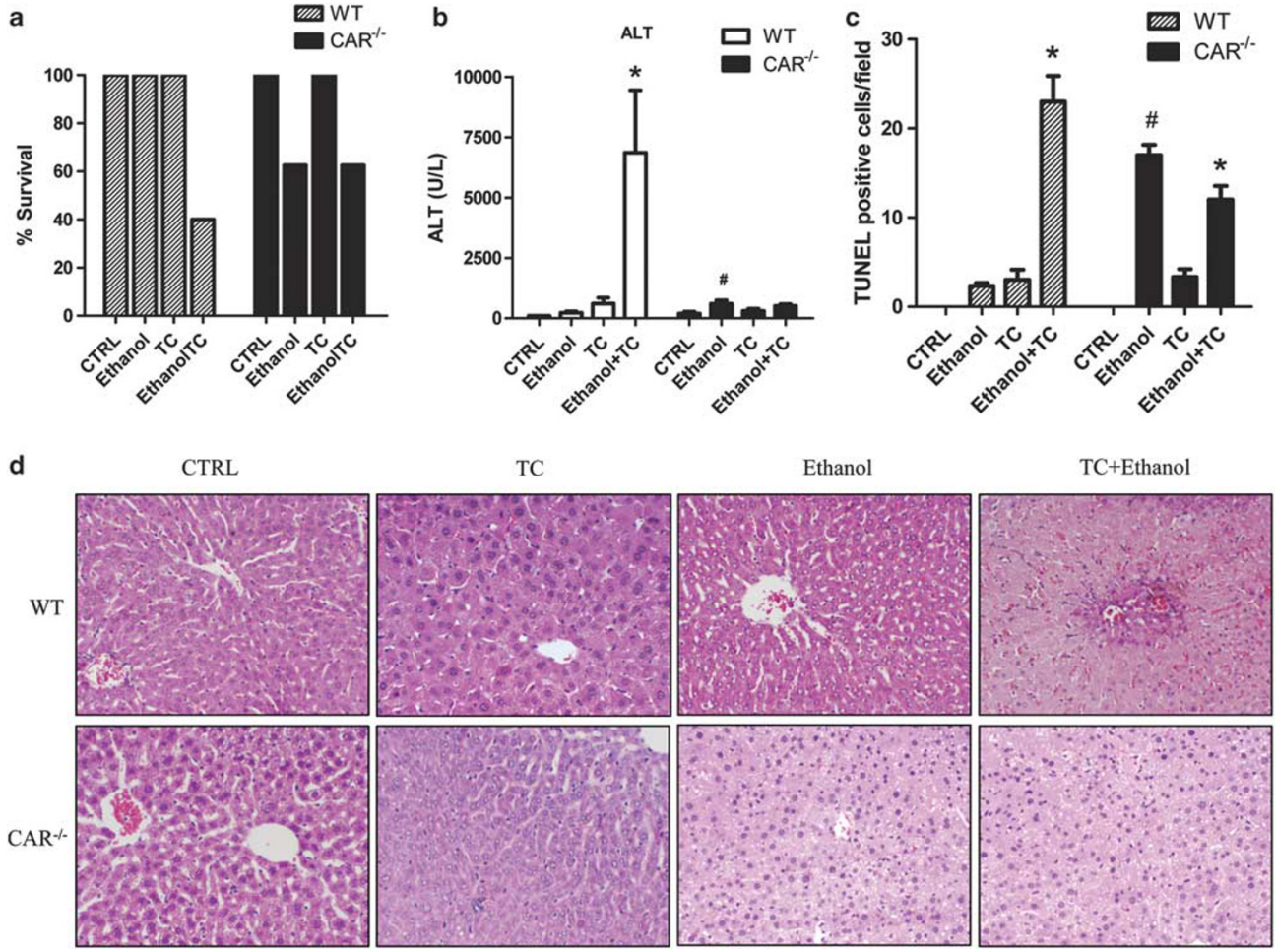

Figure 4 Synergistic effects between TCPOBOP (TC) and acute ethanol oral infusion on liver injury. (**a**) The survival rate of the wild-type and the constitutive androstane receptor ($CAR^{-/-}$) mice. The results are from at least eight mice for each group. (**b**) The serum alanine aminotransferase (ALT) levels. (**c**) Quantification of TUNEL-stained liver cells. (**d**) Representative H&E staining of the livers from the wild-type and the $CAR^{-/-}$ mice. Magnification: $\times 100$. Each group includes results from four to six mice. $^{*}P<0.05$, Ethanol + TC *vs* TC. $^{\#}P<0.05$, Ethanol *vs* CTRL.

oxygen and hydrogen peroxide. They thus form an important antioxidant defense in the liver upon alcoholic injury. We examined the expression of SOD1/2/3 and found that TCPOBOP and ethanol co-treatment strongly repressed the expression of *SOD1* and *SOD3*, but had no effect on *SOD2*, in the wild-type mice (Figures 6b–d). The expression of these two genes was also lowered in the $CAR^{-/-}$ mice treated with ethanol. Unlike the expression of the ethanol-metabolism enzyme genes, the expression of SOD1/3 in the wild-type mice was comparable to that in the $CAR^{-/-}$ mice with the

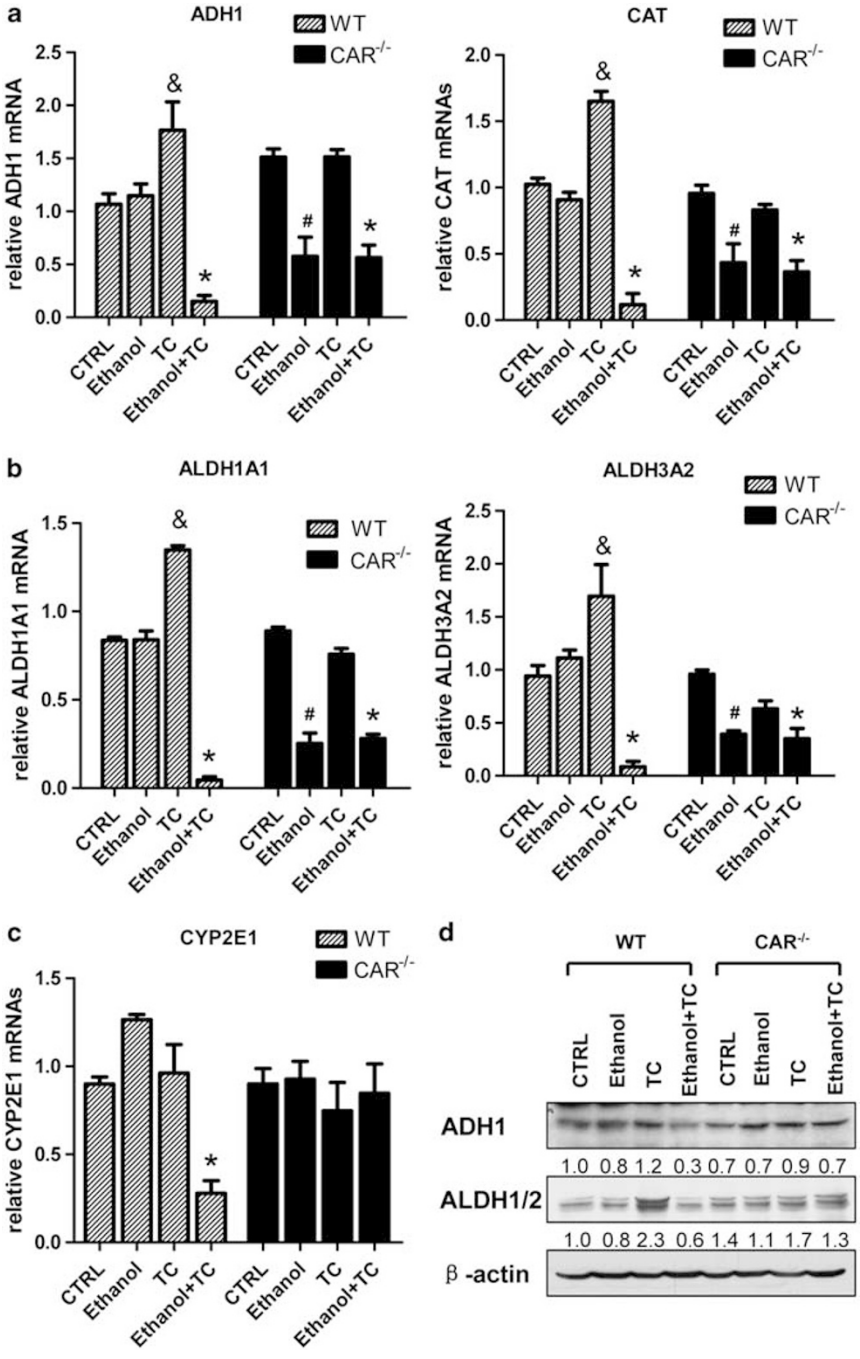

Figure 5 Gene expression analyses of liver enzymes involved in the metabolisms of ethanol after acute ethanol treatment. (**a**) Two enzymes metabolizing ethanol in the liver: alcohol dehydrogenase (ADH1) and catalase (CAT). (**b**) Two enzymes metabolizing aldehyde: ALDH1A1 and ALDH3A2. (**c**) The major enzyme in Microsomal Ethanol-Oxidizing System: CYP2E1. $^{*}P<0.05$, Ethanol + TC *vs* TC. $^{\#}P<0.05$, Ethanol *vs* CTRL; &, TC *vs* CTRL, $P<0.05$. (**d**) Western blotting analysis of ADH1 and aldehyde dehydrogenase (ALDH) expression in the liver. The band densities of ADH1 and ALDH are quantified and normalized with β-actin first and then standardized with the corresponding band densities of WT CTRL, which is normalized to 1.0.

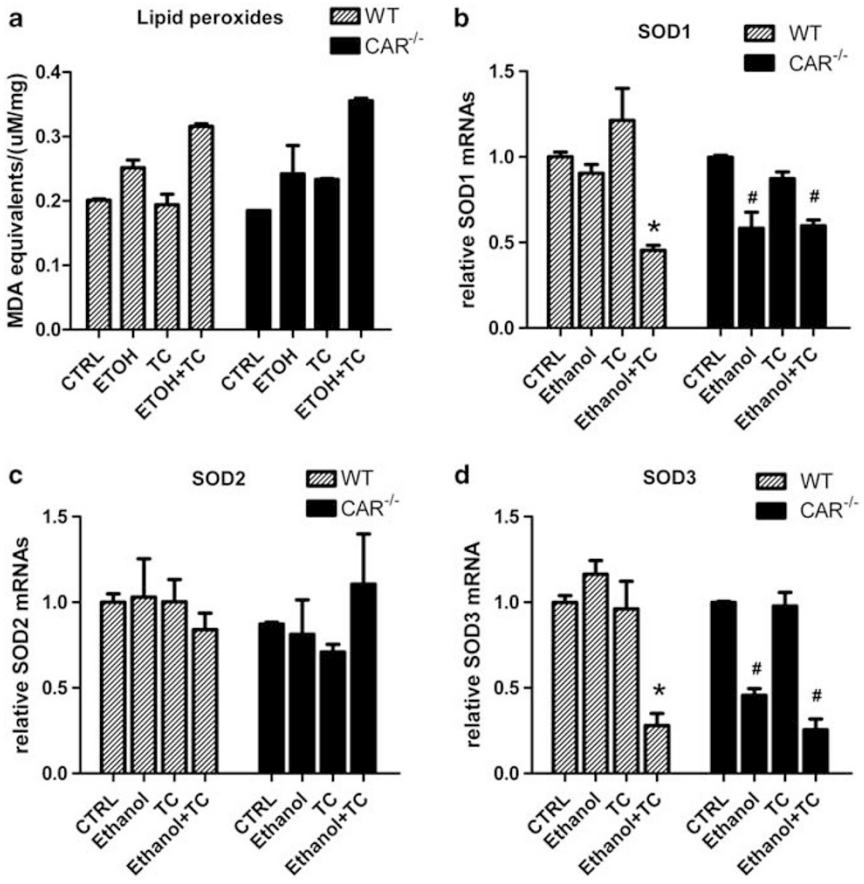

Figure 6 Oxidative stress responses in the liver exposed to acute ethanol treatment and ligand-induced constitutive androstane receptor (CAR) activation. (**a**) Levels of lipid peroxides in the livers of the mice after acute alcohol-induced liver injury. (**b–d**) mRNA levels of superoxide dismutases (SODs) in the livers. $^{*}P<0.05$, Ethanol + TC *vs* TC; $^{\#}P<0.05$, Ethanol + TC or Ethanol *vs* CTRL.

co-treatment, thus excluding the effect of SOD1/3 on alcohol-induced liver injury in this model.

DISCUSSION

CAR is essential in the detoxification of foreign substances such as prescription drugs, a variety of chemical compounds, steroid hormones, and bilirubin, all of which are ligands that activate CAR by translocating it to the nucleus, where it trans-activates or represses genes involved in metabolism of xenobiotics or endobiotics.[5,16–18] In this study, we found that ethanol could also increase the expression of CAR target genes *CYP2B10* and *CYP2E1 via* a CAR-dependent manner. However, the increase in the magnitude of CAR activation is not strong as the increase of the target gene expression is not robust. Indeed, unlike other nuclear receptors, CAR can be activated without ligands, which indicates that the basal CAR activation may be protective in liver and potentially prevent alcoholic liver disease, especially in chronic alcohol-induced liver injury. Similarly with other nuclear receptors, for example, PPARα, involved in alcoholic liver disease, CAR suppresses the steatosis in the alcohol-injured liver as more fat accumulation was seen in the $CAR^{-/-}$ mice after either the acute or the chronic treatment. In addition, the $CAR^{-/-}$ mice exhibited more susceptibility to diabetes and poor metabolism of maltose (unpublished results). Therefore, the deficiency of the $CAR^{-/-}$ mice in glucose homeostasis may also contribute to the more severe alcoholic injury in these mice. On the other hand, we and others also previously reported that CAR promotes liver regeneration and enhances the hepatocyte survival after injury.[19–21] This line of defense function of CAR may also help to promote liver repair after alcohol intake. This is consistent with our results that the $CAR^{-/-}$ mice exhibited more apoptosis and necrosis after alcohol infusion.

A surprising result we have observed is that pre-activation of CAR by TCPOBOP greatly enhances alcohol-induced liver injury *via* a CAR-dependent manner. This is reflected in the poor survival, the robust induction of ALT, and more severe necrosis and apoptosis in the wild-type but not in the $CAR^{-/-}$ mice co-treated with alcohol and TCPOBOP. This synergistic effect of ethanol and the CAR agonist may have clinical relevance to the alcoholic liver diseases. Liver has a tremendous capacity to regenerate and repair after injury. Therefore, alcohol-induced liver injury usually can be well

compromised and alcoholic liver diseases occur in only a small percentage of individuals. What is the reason that only a small population has a higher risk of alcoholic liver diseases? Considering that many prescription drugs and environment contaminants can activate CAR,[5] we propose that the simultaneous exposure to the CAR activators and alcohol consumption may greatly enhance individual susceptibilities to alcohol-induced liver damage.

The synergistic hepatotoxic effects of ethanol and TCPOBOP might be attributed to the repression of expression of key genes involved in the metabolisms of ethanol. These genes include *ADH1*, *CAT*, *ALDH1A1*, and *ALDH3A2*. Notably, TCPOBOP treatment alone significantly enhances the expression of these enzymes, which is absent in $CAR^{-/-}$ mice, implying that CAR may potentially upregulate the expression of these genes. However, the combination of TCPOBOP and ethanol greatly suppresses their expression, to the even lower extent than that in the $CAR^{-/-}$ mice treated with both TCPOBOP and ethanol. It seems that ethanol converts TCPOBOP into an inverse CAR agonist for these target genes. Future efforts will be spent on confirming and characterizing the inverse agonist effects of TCPOBOP after ethanol treatment.

Another interesting question raised from this study is that whether PXR is also involved in alcoholic liver disease. PXR is also involved in liver repair and lipid and glucose metabolism.[22,23] Furthermore, CAR and PXR coordinate with each other as xenobiotics sensors and regulates expression of cytochrome P450 enzymes and detoxification of xenobiotics.[24] The functional overlapping and crosstalk of these two nuclear receptors have been systematically analyzed and reviewed. Therefore, it may be interesting to use similar strategy with the one applied in this study to characterize the roles of PXR and its cooperation with CAR in alcoholic liver diseases.

Our results may also contribute to the understanding of correlation between alcohol consumption and liver cancer. It has been shown that the synergistic effects of CAR activation and chemical carcinogen lead to hepatocellular tumorigenesis.[20,25] Ethanol is a well-known promoter of liver cancer, therefore, chronic alcohol consumption and frequent exposure to chemicals or drugs that activate CAR potentially promote the development of liver cancer.

In summary, we concluded that the basal CAR activity may protect the liver from alcohol-induced injury. However, preactivation of CAR will greatly enhance alcohol-induced liver injury by repressing the expression of alcohol-metabolizing enzymes. Alcohol consumption in the context of CAR activation by chemicals such as prescription drugs or CAR-activating environmental contaminants needs to be avoided to prevent liver damage.

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DISCLOSURE/CONFLICT OF INTEREST
The authors declare no conflict of interest.

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